REMARKS

Claims 41 - 55 are pending in the instant application. Claims 41, 45, 47, 48 and 50 have been amended, and claims 54 and 55 have been added by the present amendment. The claims have been amended to further clarify the claim language, and Applicants assert the amendments are not narrowing and have not been made to overcome prior art. In general, the claims conform the terms used to recite the non-reverting Bacillus host, that is " a mutant alkalophilic Bacillus host or strain"; to clarify that the mutant alkalophilic Bacillus host includes a chromosomal deletion of the gene encoding the wild-type protein; and that it is the gene encoding the wild-type alkaline protease has been deleted as opposed to the enzyme. Support may be found in general in the specification and specifically at page 7, lines 28 - 38 of the specification. New independent claim 54 is directed to a method of producing a mutant high alkaline protease by transforming a Bacillus novo species PB92 or a derivative thereof which is incapable of producing a wild-type alkaline protease with an integration cassette comprising a gene encoding a mutant alkaline protease. Claim 55 is direct to specific mutations in the alkaline protease. Support is found through out the specification and in particualr in the examples.

The Examiner has rejected claims 41 - 53 under 35 U.S.C. §112, first and second paragraphs, and Applicants respectfully traverse said rejections. There are no cited prior art rejections.

Rejection under 35 U.S.C. §112, first paragraph:

The Examiner states,

> ..."While being enabling for the wild-type alkaline protease of Bacillus novo species PB92, the gene encoding said wild-type alkaline protease of Bacillus novo species PB92, and a gene encoding a mutant alkaline protease comprising a nucleotide sequence consisting of the gene encoding the wild-type alkaline protease of Bacillus novo species PB92 having the codon for M216 replaced with a codon coding for Q, the codon for S160 replaced by the codon coding for D, or the codon of N212 replaced with the codon coding for D; does not reasonably provide enablement for any wild-type alkaline protease or any gene encoding any mutant alkaline protease.

The Examiner also states,

> "the amount of experimentation to determine the specific wild-type alkaline protease and the specific mutation in the claimed gene encoding the mutant alkaline protease is enormous. .... the Examiner finds that one skilled in the art would require additional guidance, such as information regarding the specific wild-type alkaline protease and the specific type of mutation in the claimed gene encoding a mutant alkaline protease. Without such a guidance, the experimentation left to those skilled in the art is undue."

Applicants submit that the specification does enable the instant claims and further teaches; 1) high alkaline protease genes and wild type alkaline protease genes at page 10, lines 21-32; page 12, lines 8-21; and in Example 3; 2) mutant proteases at page 13, lines 1-16; and mutation of a protease gene in Example 3; 3) cloning and transformation techniques at page 9, lines 26-38 through page 10, lines 1-2; page 13, lines 17-38; page 14, lines 1-38; and in particular, transformation techniques for alkalophilic Bacillus strains are disclosed at page 14, lines 23-25; and 4) production of non-reverting alkalophilic Bacillus strain hosts at page 7, lines 17- 38 through page 9, lines 1-26; page 10, lines 3-10; and page 12, lines 31-33.

Furthermore, it is well established that a patent need not disclose what is well known in the art and available to the public. Applicants submit that high alkaline proteases, as well as the tools necessary for mutating, cloning and transforming genes were well within the skill of those in the art as of the filing date of the present invention. In particular, techniques for transforming alkalophilic Bacillus host cells were known, see for example EP-A-0283075.

Additionally, Applicants submit that a variety of high alkaline proteases were known by those of skill in the art as of the filing date of the instant application.

High alkaline proteases were described in USP 3,723,250 (cited in the specification) ; USP 4,480,037 (cited in the specification); USP 3,905,869; and USP 4,052,262.

Additionally a preferred group of mutant proteases is disclosed at the bottom of page 12 and bridging page 13 of the specification. It is stated "Preferably the polypeptide is a mutant high alkaline protease, most preferably the mutant protease is one described in EPA 0328229 and WO89/06279 and capable of production on an industrial scale. For example with respect to EPA 0328229, it is taught that preferred mutations of PB92 protease include positions 60, 94, 97, 102, 105, 116, 123 - 128, 150,

152, 153, 160, 183, 203, 211, 212, 213, 214, and 216. Particularly preferred positions are disclosed as positions 116, 126, 127, 128, 160, 166, 169, 212, and 216. While Applicants specifically made mutations at positions 160, 212 and 216 of Bacillus novo species PB92, the claims should not be limited to said mutations.

Examples of alkalophilic Bacillus strains and the proteases derived from them are also described in Hiroski (1982, Alkalophilic Microorganisms, Springer Verlag, New York). Furthermore, Grant et al. (1990, FEMS Microbiology Reviews 75: 255-270) disclose at page 263 commercial products which comprise alkaline proteases obtainable from alkalophilic Bacillus. Pack et al. (1986, Korean Journal of Applied Microbiology and Bioengineering, vol. 14: 517) disclose the cloning of a protease gene from Bacillus K17. Takii et al. (1990, *Appl. Microbiol. Biotechnol.* 34:57-62) disclose alkaline serine protease obtainable from Bacillus alkalophilic subsp. halodurans KP1239, and present a characterization of the protease including molecular weight and N-terminal amino acid sequence.

Applicants submit that obtaining the nucleic acid sequence encoding an alkaline protease obtainable from characterized or deposited microorganisms would be deemed well within the skill of the art, especially when characteristics such as the microorganism from which the protease is obtained, molecular weight, and sequence information are available.

Rejection under 35 U.S.C. §112, second paragraph:

The Examiner has rejected claims 41 - 53 because it is argued that the term "high" with respect to "a mutant high alkaline protease" is a relative term and it is not known what pH range is considered to be "high alkaline".

Applicants assert the term, taken with the teaching in the specification is definite. At page 10 of the specification it is taught,

> "Of particular interest in the present invention is the production of proteases derived from alkalophilic *Bacilli*. These protease are referred to herein as high alkaline proteases. As a host cell for the expression of mutated genes encoding modified or so-called protein engineered proteins it is preferable to use cells in which the genes are structurally expressed at a high level. Therefore, for the production of high alkaline proteases, preferably alkalophilic *Bacillus* strains are used in host cells.
> High alkaline protease producing *Bacilli* are taxonomically not well classified and are generally referred to as alkalophilic *Bacillus*

> strains. For the present invention alkalophilic bacilli are defined as *Bacillus* strains that grow under alkaline conditions, generally pH 9 - 11 (Horikoshi, and Akiba, (1982), *Alkalophilic microorganisms*, Springer Verlag, New York). The alkaline proteases produced by such *Bacilli* are called high alkaline protease. Examples of *Bacillus* strains capable of growing at alkaline pH are described in, for example, U.S. Patent Nos. 3,723,250, RE 30,602 and 4,480,037."

The Examiner has also rejected the claims indicating "it is not known what is the specific mutation of the claimed "mutant high alkaline protease". Applicants assert it is not required to delineate the specific mutation and that the phrase "mutant high alkaline protease" is clear and definite and is explicitly described in the specification, at page 13, lines 8-16. As is stated in the instant specification:

> "Where it is desired to obtain a mutated protease, the DNA sequence can be mutated so that at least one amino acid is different from the wild type protease. More than one amino acid may be mutated so long as the resulting peptide maintains the capacity to degrade. In some instances the reaction rate may be lower than that of the native protease; or it may be the same or greater reaction rate as native protease, depending upon the desired application."

Applicants submit that one of skill in the art would have an immediate understanding of the phrase "mutated high alkaline protease" from the teachings of the specification. According to the teachings of the specification, the "result" of any mutation places no limitation on the claimed invention, the reaction rate may be lower, the same or higher than the native protease, depending upon the desired application.

As Applicants have pointed out various high alkaline proteases, as well various alkalophilic Bacillus, are known in the art. Cloning and transformation techniques are deemed routine to those of skill in the art. The preparation of mutants of proteases are well within the skill in the art.

Applicants believe the currently pending claims comply with the requirements of section 112 first and second paragraphs and respectfully request the withdrawal of all

rejections under 35 U.S.C. §112. Applicants submit that this application which has been pending for over ten years is in condition for allowance. Such action by the Examiner is earnestly solicited.

Respectfully submitted,

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MARKED-UP VERSION OF AMENDED CLAIMS

41.(Once Amended) A method for the production of a mutant high alkaline protease, said method comprising the steps of:

a) obtaining a non-reverting **mutant** alkalophilic Bacillus host incapable of producing a wild-type high alkaline protease, wherein said **mutant alkalophilic** Bacillus host comprises **a chromosomal deletion of the gene encoding the wild-type alkaline protease and** an integration cassette comprising a gene encoding **[said] a** mutant high alkaline protease; and

b) growing said **mutant alkalophilic** Bacillus host under conditions whereby said mutant high alkaline protease is expressed.

45.(Once Amended) The method of Claim 41 wherein said **[wild-type protease] gene encoding the wild-type alkaline protease in said mutant alkalophilic Bacillus host** has been deleted by homologous or illegitimate recombination.

47.(Once amended) The method of Claim 41 wherein said integration cassette is integrated into the genome of said **mutant** alkalophilic Bacillus host.

48.(Once amended) A method of obtaining a non-reverting **mutant** alkalophilic Bacillus strain having a reduced level of a **wild-type [extracellular]** high alkaline protease, said method comprising the steps of:

a) transforming an alkalophilic Bacillus strain comprising a **gene encoding the** wild-type alkaline protease **[gene]** with a cloning vector comprising DNA encoding a replication function and 5' and 3' flanking non-coding regions of said **gene encoding the** wild-type high alkaline protease **[gene]** but not the coding region of said **gene encoding the** wild-type high alkaline protease gene, wherein a sufficient amount of said 5' and 3' flanking non-coding regions is present to provide for homologous recombination with **[said wild-type high alkaline protease gene] the indigenous gene encoding the wild-type alkaline protease of said alkalophilic Bacillus strain** whereby transformants having a reduced level of **[high alkaline protease] said wild-type alkaline protease** are obtained;

b) growing said transformants under conditions whereby the replication function encoded by said cloning vector is inactivated; and

c) isolating transformants having a reduced **[extracellular alkaline protease]** level **of the wild-type alkaline protease**.

50.(Once amended) **[An] A mutant** alkalophilic Bacillus strain producing a mutant high alkaline protease and no detectable **level of a** wild-type **[extracellular]** high alkaline protease, wherein said **mutant alkalophilic** Bacillus strain is obtained by growing an alkalophilic Bacillus strain which is incapable of producing said wild-type high alkaline protease transformed with a plasmid expression vector comprising said mutant high alkaline protease gene.